

IN THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claims 1-24 (previously cancelled).

25. (Currently Amended) A method of modifying a lysosomal hydrolase comprising contacting said lysosomal hydrolase hydrolases with an isolated N-acetylglucosamine-1-phosphotransferase, which has a specific activity of at least 10^6 pmol/h/mg to produce a modified lysosomal hydrolase.

26. (Previously presented) The method of Claim 25, further comprising purifying said modified lysosomal hydrolase after said contacting.

27. (Previously presented) The method of Claim 25, wherein said N-acetylglucosamine-phosphotransferase catalyzes the transfer of N-acetylglucosamine-1-phosphate from UDP-N-Acetylglucosamine to a mannose on the hydrolase.

28. (Previously presented) The method of Claim 25, wherein said lysosomal hydrolase is a recombinant hydrolase.

29. (Previously presented) The method of Claim 25, wherein said lysosomal hydrolase is selected from the group consisting of α -glucosidase, α -iduronidase, α -galactosidase A, arylsulfatase, N-acetylgalactosamine-6-sulfatase, β -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, B-glucuronidase, Heparan N-sulfatase, N-Acetyl- α -glucosaminidase, Acetyl CoA- α -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid β -galactosidase G_{M1} Ganglioside, Acid β -galactosidase, Hexosaminidase A, Hexosaminidase B, α -fucosidase, α -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase,

Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and
Glucocerebrosidase β -Glucosidase.

30. (Currently Amended) The method of Claim 25, further comprising contacting said modified lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase, which catalyzes the removal of N-acetylglucosamine from said modified lysosomal hydrolase hydrolases and generates a terminal mannose 6-phosphate on said hydrolase.

31. (Previously presented) The method of Claim 25, wherein said N-acetylglucosamine-1-phosphotransferase has a specific activity of at least 5×10^6 pmol/h/mg.

32. (Previously presented) The method of Claim 25, wherein said N-acetylglucosamine-1-phosphotransferase has a specific activity of at least 12×10^6 pmol/h/mg.

33. (Previously presented) The method of claim 25, wherein the N-acetylglucosamine-1-phosphotransferase comprises an α subunit, a β subunit, and a γ subunit; and wherein the α and β subunits are encoded by a DNA molecule comprising SEQ ID NO:20; and the γ subunit is encoded by a DNA molecule comprising nucleotides 96 to 941 of SEQ ID NO:5.

34. (Previously presented) The method of claim 25, wherein the N-acetylglucosamine-1-phosphotransferase comprises an α subunit, a β subunit, and a γ subunit; and wherein the α and β subunits are encoded by a DNA molecule which hybridizes under stringent conditions to the complement of SEQ ID NO:20; and the γ subunit is encoded by a DNA molecule which hybridizes under stringent conditions to the complement of nucleotides 96 to 941 of SEQ ID NO:5; wherein the combination of the α subunit, a β subunit, and a γ subunit yields a protein with the activity to catalyze the transfer of N-acetylglucosamine-1-phosphate from UDP-N-Acetylglucosamine to a mannose on the hydrolase.

35. (Previously presented) The method of Claim 25, wherein the lysosomal hydrolase is α -glucosidase.

36. (Previously presented) The method of Claim 25, wherein the lysosomal hydrolase is α -iduronidase.

37. (Previously presented) The method of Claim 25, wherein the lysosomal hydrolase is α -galactosidase A.

38. (Previously presented) A modified lysosomal hydrolase produced by the method of Claim 25.

39. (Currently Amended) A method of preparing a phosphorylated lysosomal hydrolase comprising contacting said lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase, which has a specific activity of at least about 472,000 units/mg and which catalyzes the removal of N-acetylglucosamine from said modified lysosomal hydrolase hydrolases and generates a terminal mannose 6-phosphate on said hydrolase, and wherein said lysosomal hydrolase comprises a N-acetylglucosamine phosphomannose diester.

40. (Previously presented) The method of Claim 39, wherein said method further comprises purifying the phosphorylated lysosomal hydrolase.

41. (Previously presented) The method of Claim 39, wherein said lysosomal hydrolase is selected from the group consisting of α -glucosidase, α -iduronidase, α -galactosidase A, arylsulfatase, N-acetylgalactosamine-6-sulfatase, β -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, B-glucuronidase, Heparan N-sulfatase, N-Acetyl- α -glucosaminidase, Acetyl CoA- α -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid β -galactosidase G_{M1} Ganglioside, Acid β -galactosidase, Hexosaminidase A, Hexosaminidase B, α -fucosidase, α -

N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase β -Glucosidase.

42. (Currently Amended) The method of Claim 39, wherein said N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase α -N-Acetylglucosaminidase catalyzes the removal of N-acetylglucosamine from N-acetylglucosamine phosphomannose diester to generate a terminal mannose 6-phosphate on said lysosomal hydrolase.

Claims 43 and 44. (Cancelled)

45. (Currently Amended) The method of Claim 39, wherein the N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase α -N-Acetylglucosaminidase is encoded by a DNA molecule comprising nucleotides 151 to 1548 of SEQ ID NO:7.

46. (Currently Amended) The method of Claim 39, wherein the N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase α -N-Acetylglucosaminidase is encoded by a DNA molecule which hybridizes under stringent conditions to the complement of nucleotides 151 to 1548 of SEQ ID NO:7.

47 (Previously presented) The method of Claim 39, wherein the lysosomal hydrolase is α -glucosidase.

48. (Previously presented) The method of Claim 39, wherein the lysosomal hydrolase is α -iduronidase.

49. (Previously presented) The method of Claim 39, wherein the lysosomal hydrolase is α -galactosidase A.

50. (Currently Amended) A method of preparing a phosphorylated lysosomal hydrolase comprising:

contacting said lysosomal hydrolase with an isolated N-acetylglucosamine-phosphotransferase, which has a specific activity of at least 10^6 pmol/h/mg to produce a modified lysosomal hydrolase; and

contacting said modified lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase, which catalyzes the removal of N-acetylglucosamine from said modified lysosomal hydrolase hydrolases and generates a terminal mannose 6-phosphate on said hydrolase.

51. (Previously presented) The method of Claim 50, further comprising purifying said phosphorylated lysosomal hydrolase after said contacting with the isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase.

52. (Previously presented) The method of Claim 50, further comprising purifying said modified lysosomal hydrolase prior to said contacting with the isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase.

53. (Previously presented) The method of Claim 50, wherein said lysosomal hydrolase is selected from the group consisting of α -glucosidase, α -iduronidase, α -galactosidase A, arylsulfatase, N-acetylgalactosamine-6-sulfatase, β -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, B-glucuronidase, Heparan N-sulfatase, N-Acetyl- α -glucosaminidase, Acetyl CoA- α -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid β -galactosidase G_{M1} Ganglioside, Acid β -galactosidase, Hexosaminidase A, Hexosaminidase B, α -fucosidase, α -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase β -Glucosidase.

54. (Previously presented) The method of Claim 50, wherein said N-acetylglucosamine-1-phosphotransferase has a specific activity of at least 5×10^6 pmol/h/mg.

55. (Previously presented) The method of Claim 50, wherein said N-acetylglucosamine-1-phosphotransferase has a specific activity of at least 12×10^6 pmol/h/mg.

56. (Previously presented) The method of Claim 50, wherein the phosphorylated lysosomal hydrolase comprises at least 6% bis-phosphorylated oligosaccharides.

57. (Previously presented) The method of Claim 50, wherein the phosphorylated lysosomal hydrolase comprises at least 100% bis-phosphorylated oligosaccharides.

58. (Previously presented) The method of Claim 50, wherein the phosphorylated lysosomal hydrolase comprises at least 5 mannose 6-phosphates.

59. (Previously presented) The method of claim 50, wherein the N-acetylglucosamine-1-phosphotransferase comprises an α subunit, a β subunit, and a γ subunit; and wherein the α and β subunits are encoded by a DNA molecule comprising SEQ ID NO:20; and the γ subunit is encoded by a DNA molecule comprising nucleotides 96 to 941 of SEQ ID NO:5.

60. (Previously presented) The method of Claim 50, wherein the N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase is encoded by a DNA molecule comprising nucleotides 151 to 1548 of SEQ ID NO:7.

61. (Previously presented) The method of claim 50, wherein the N-acetylglucosamine-1-phosphotransferase comprises an α subunit, a β subunit, and a γ subunit; and wherein the α and β subunits are encoded by a DNA molecule which hybridizes under stringent conditions to the complement of SEQ ID NO:20; and the γ subunit is encoded by a DNA molecule which hybridizes under stringent conditions to the complement of nucleotides 96 to 941 of SEQ ID NO:5; wherein the combination of the α subunit, a β subunit, and a γ

subunit yields a protein with the activity to catalyze the transfer of N-acetylglucosamine-1-phosphate from UDP-N-Acetylglucosamine to a mannose on the hydrolase.

62. (Previously presented) The method of Claim 50, wherein the N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase is encoded by a DNA molecule which hybridizes under stringent conditions to the complement of nucleotides 151 to 1548 of SEQ ID NO:7.

63. (Previously presented) The method of Claim 50, wherein the lysosomal hydrolase is α -glucosidase.

64. (Previously presented) The method of Claim 50, wherein the lysosomal hydrolase is α -iduronidase.

65. (Previously presented) The method of Claim 50, wherein the lysosomal hydrolase is α -galactosidase A.

66. (Previously presented) A phosphorylated lysosomal hydrolase produced by the method of Claim 50.

67. (Previously presented) A composition comprising the modified lysosomal hydrolase of Claim 38 and a carrier.

68. (Previously presented) A composition comprising the phosphorylated lysosomal hydrolase produced by the method of Claim 50.

69. (Previously presented) The method of Claim 30, wherein said isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase has a specific activity of at least about 1000 units/mg.

70. (Previously presented) The method of Claim 30, wherein said isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase has a specific activity of at least about 472,000 units/mg.

71. (Previously presented) The method of Claim 50, wherein said isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase has a specific activity of at least about 1000 units/mg.

72. (Previously presented) The method of Claim 50, wherein said isolated N-acetylglucosamine-1-phosphodiester α -N-Acetylglucosaminidase has a specific activity of at least about 472,000 units/mg.